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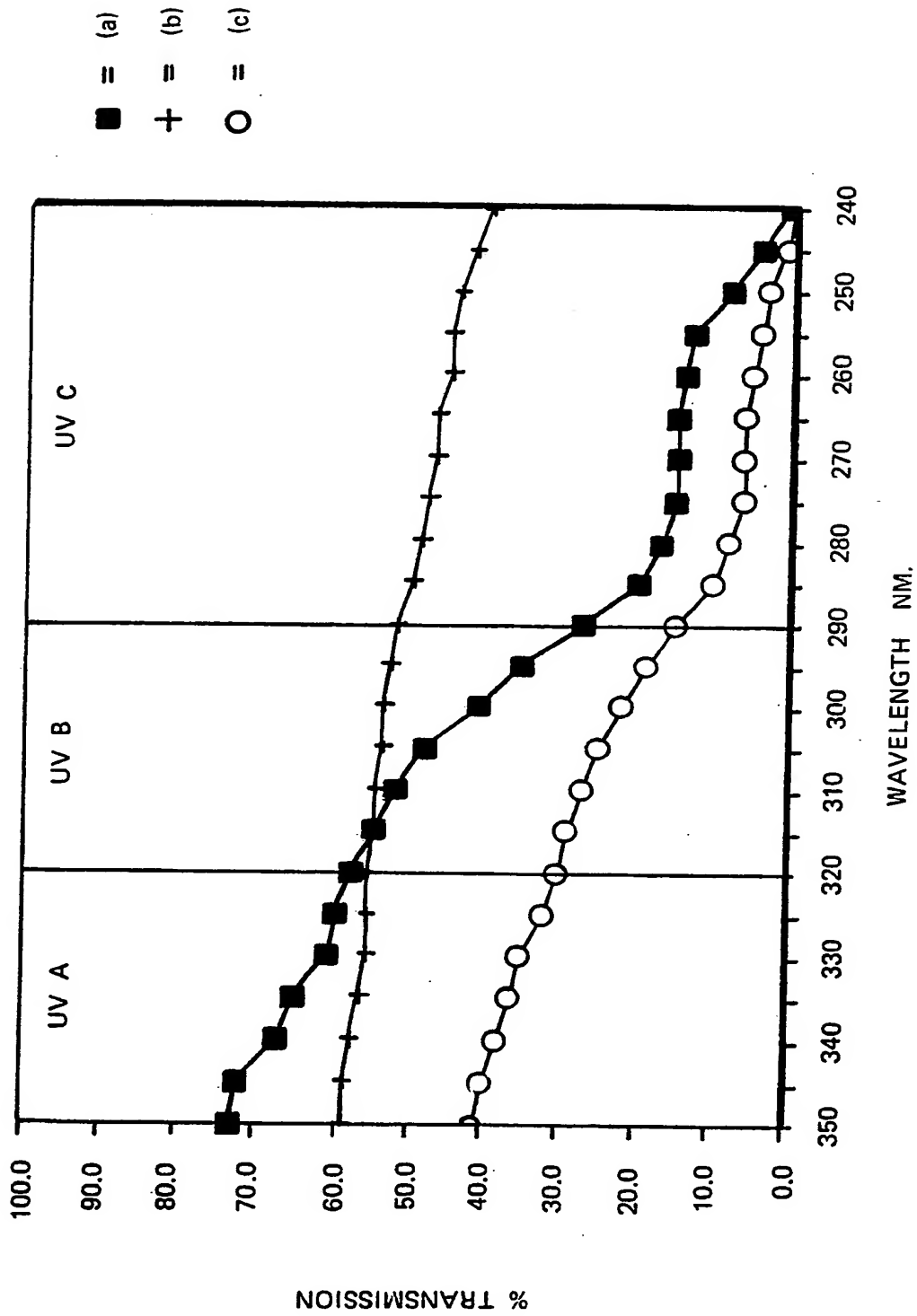
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(54) UV absorption agents

(57) The invention relates to the use of melanin in the form of natural melanin granules as a sunscreen agent. In a preferred embodiment the pigment Eu-melanin is used and preferably the granules are greater than 1 μ in diameter. The agent is preferably employed with a suitable carrier as an ointment, cream or gel.

The invention also provides for the use of keratin and the hydrolysis products therefrom to be used as a sunscreen agent.

Fig 1.

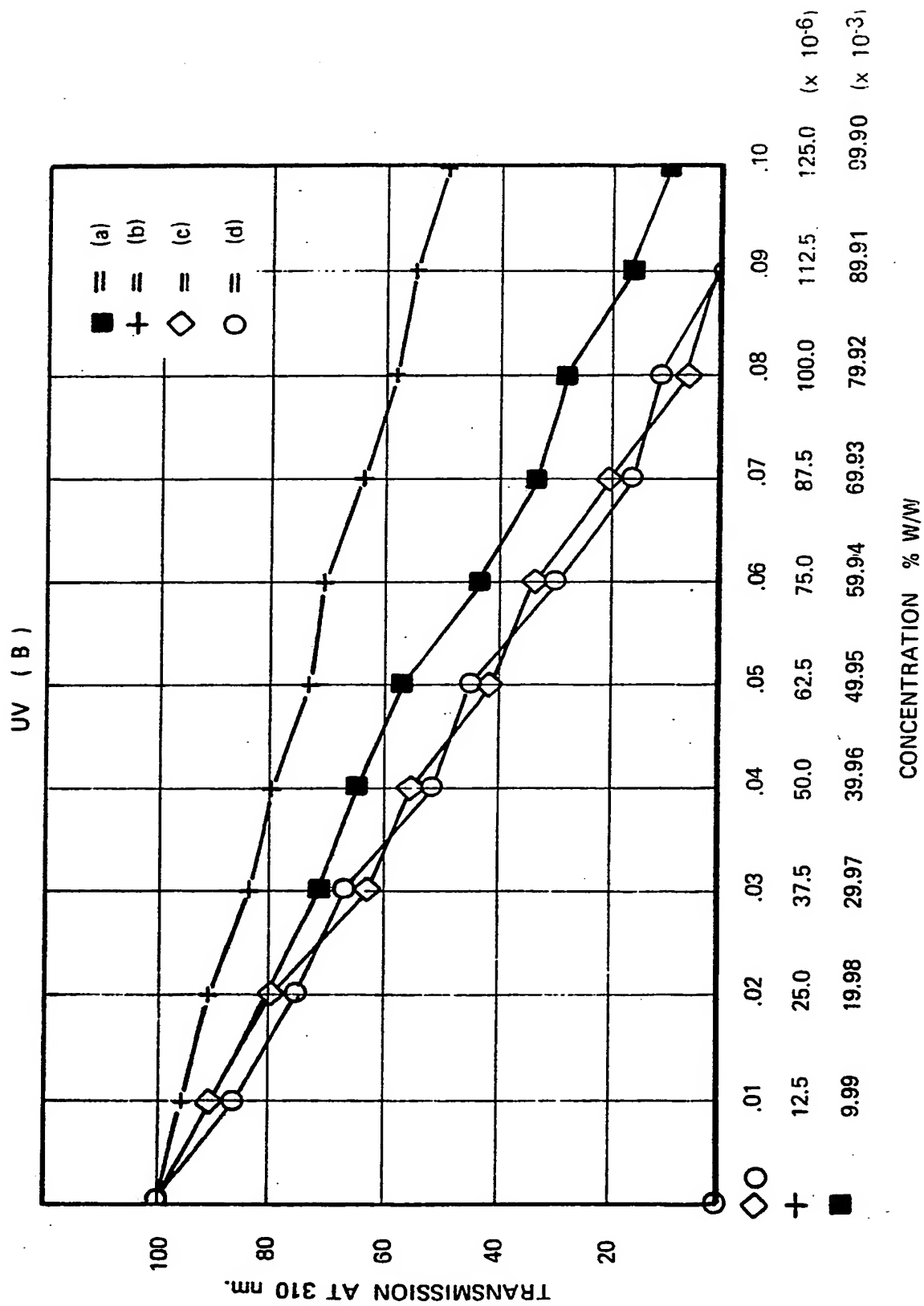


Fig 2.

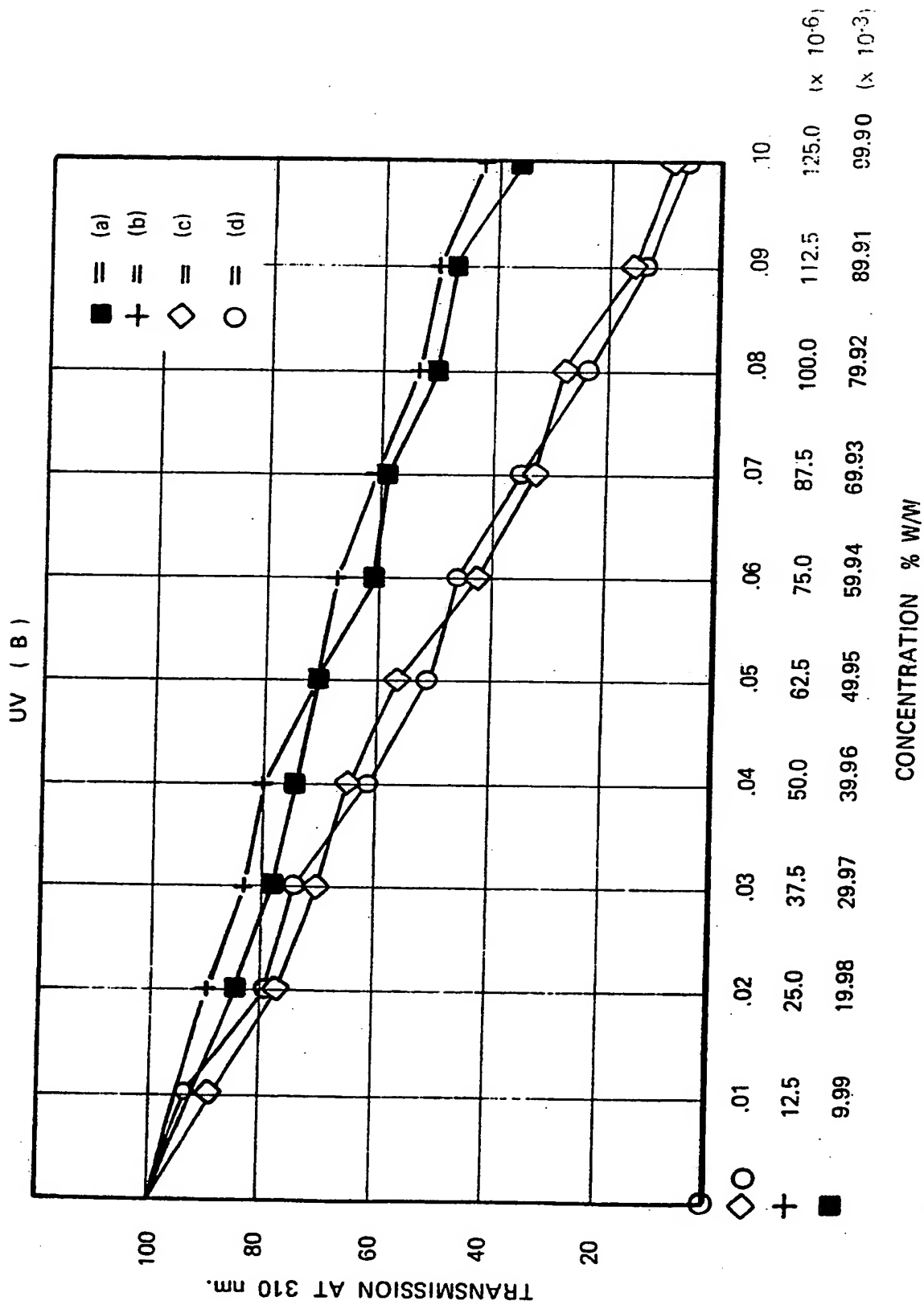


Fig 3.

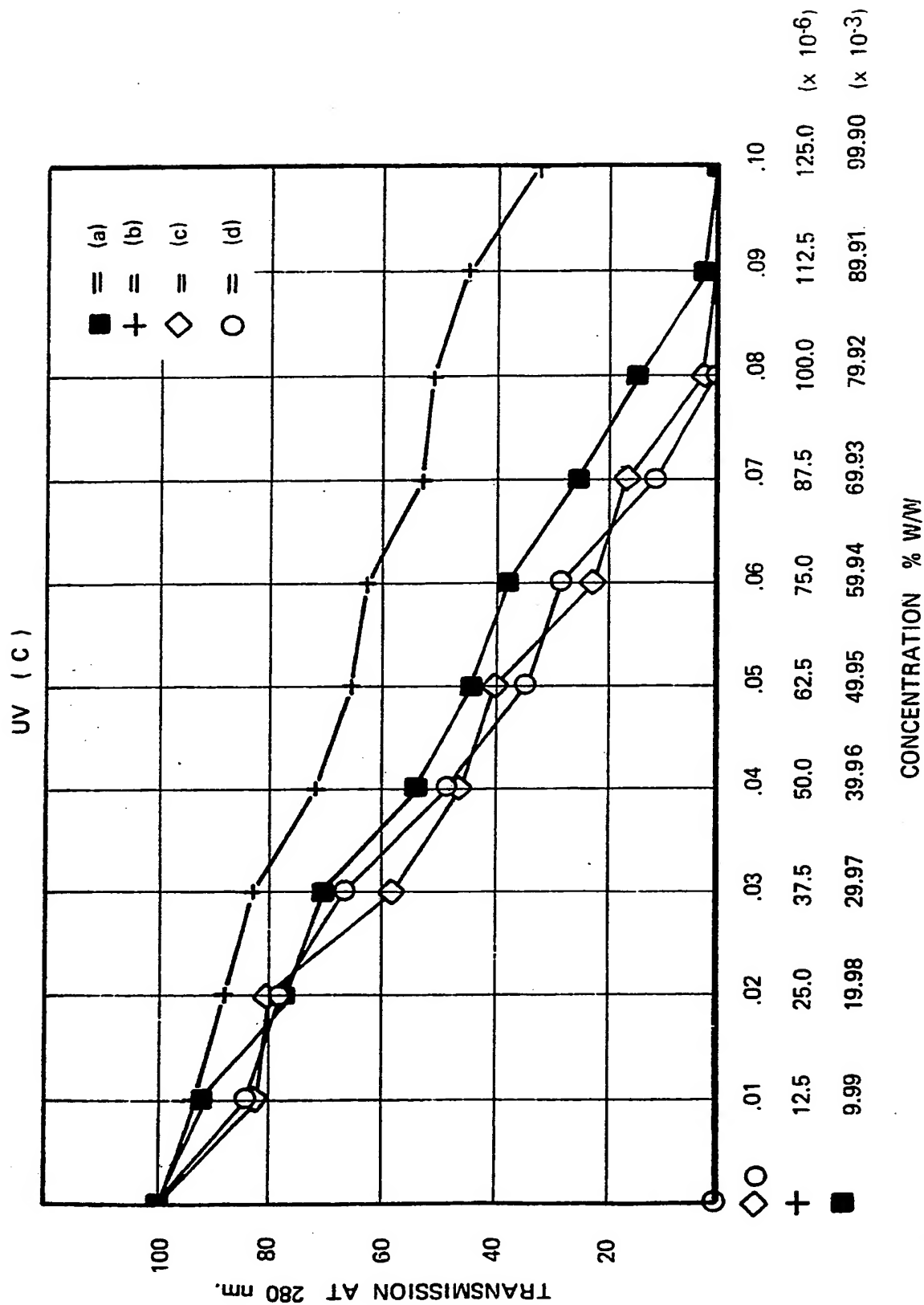


Fig 4.

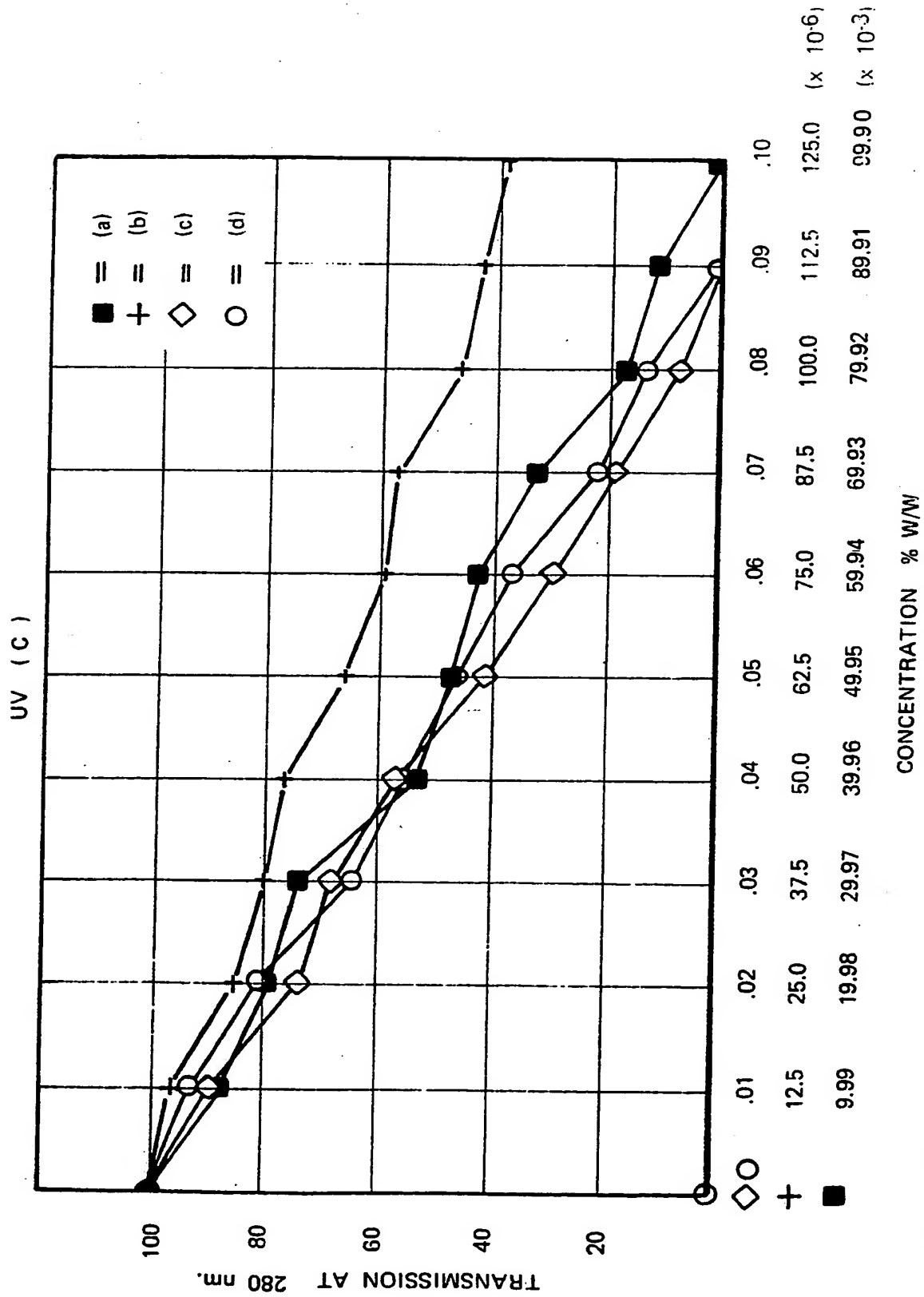


Fig 5.

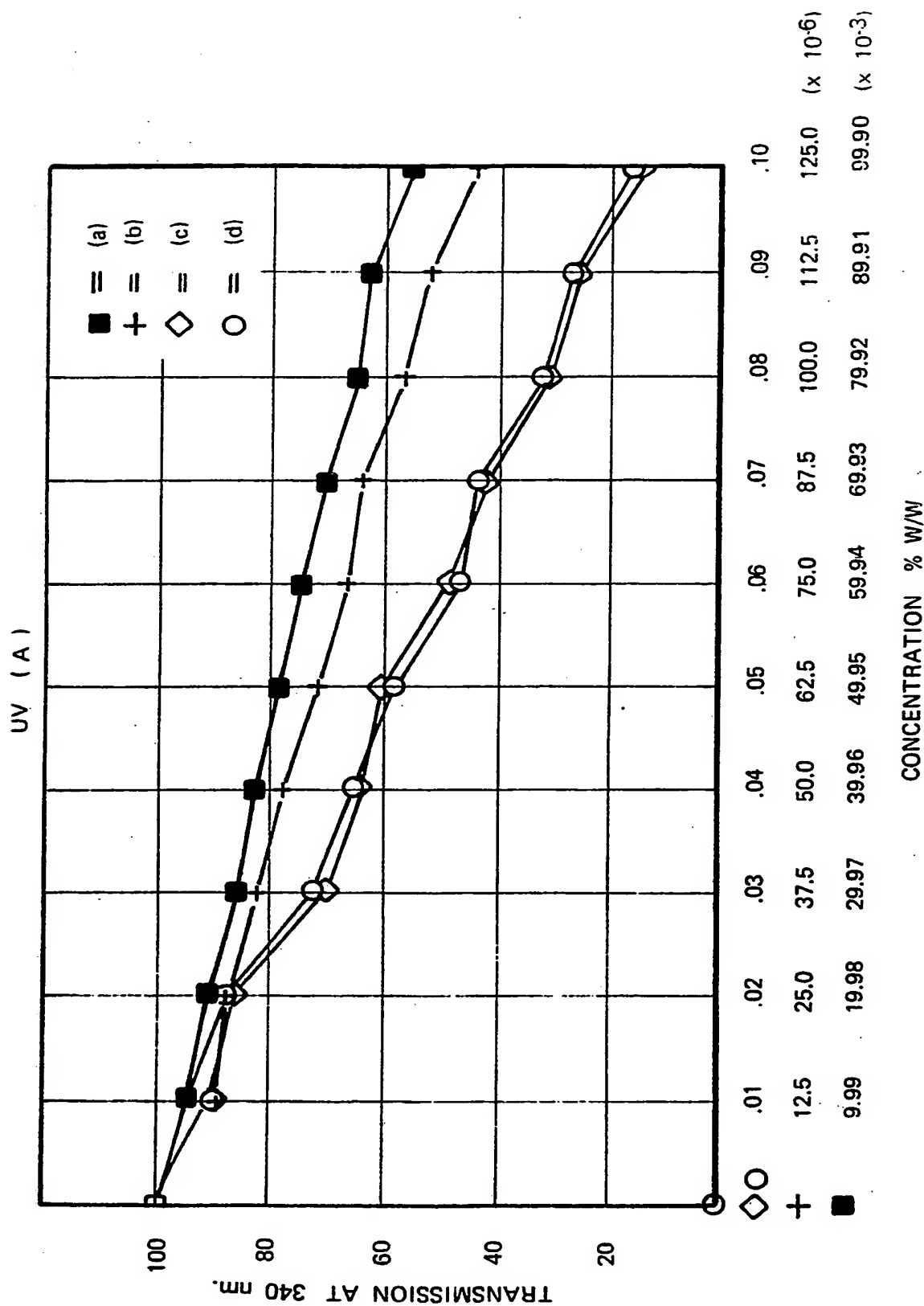


Fig 6.

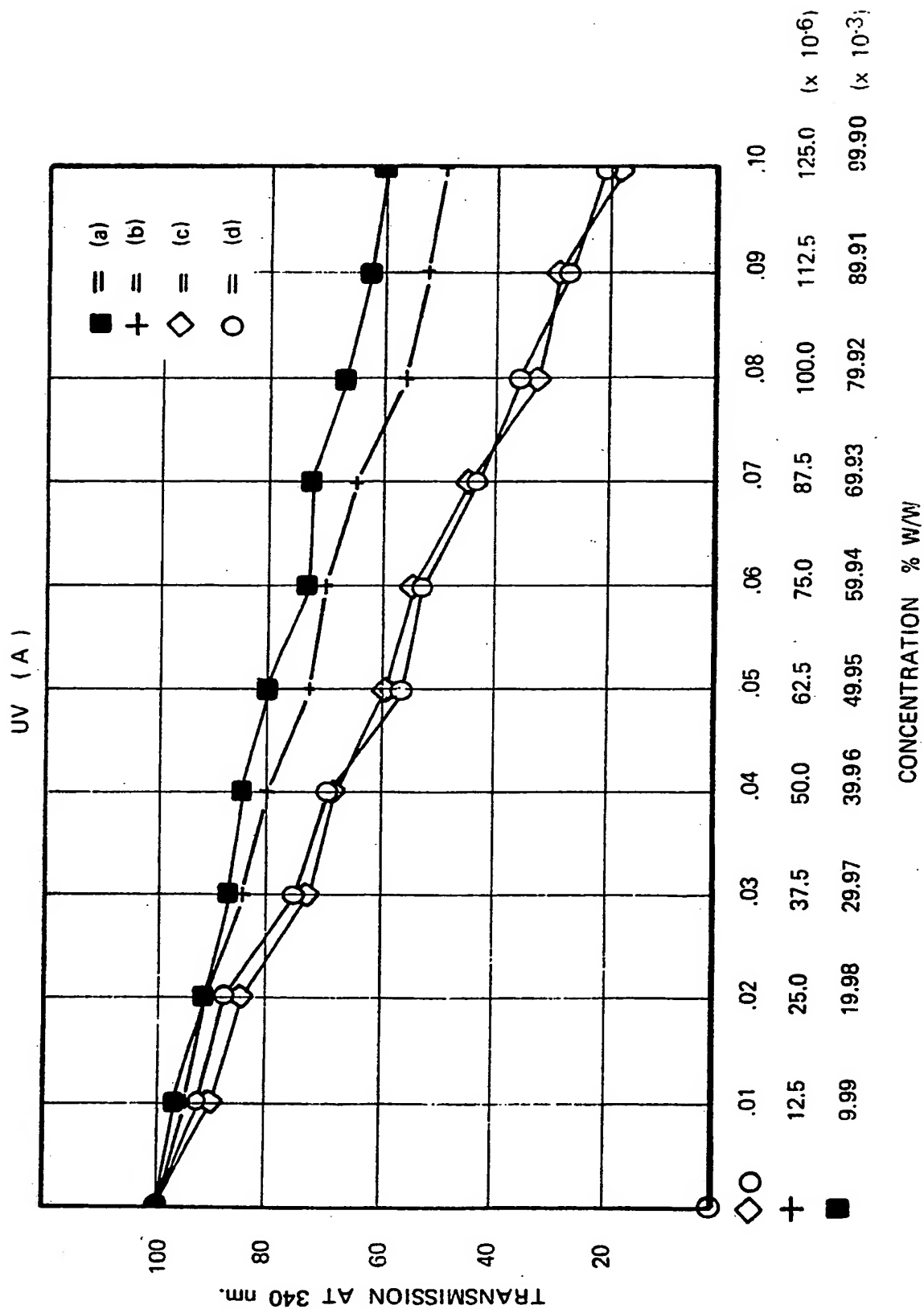


Fig 7.

DESCRIPTIONU.V. ABSORPTION AGENTS

The present invention relates to U.V. absorbing agents and compositions thereof and more particularly, though not exclusively, to U.V. absorption agents and compositions thereof which are unlikely to cause allergic response when applied to the body.

Erythema of the skin, skin cancer and associated diseases are becoming major health problems, and so effective screen agents, against the causative agents, i.e. e.m. radiation in the region 200 to 800 nm, and more particularly U.V. radiation in the region 200 to 400 nm, have been sought.

The current screen agents used are synthetic, for example, allantoin combined with aminobenzoid acid (allantoin p-aminobenzoic acid complex), aminobenzoic acid (p-aminobenzoic acid) cinoxate, diethanolamine p-methoxycinnamate (p-methoxycinnamic acid diethanolamine), digalloyl trioleate, 5-(3,3-dimethyl 2 norbornyliden)-3-penten-2-one, dioxybenzone, dipropylene glycol salicylate, ethyl 4-(bis (hydroxypropyl)) aminobenzoate (propoxylate of p-aminoethylbenzoate) 2-ethylhexyl 2-cyano-3, 3-diphenylacrylate, ethylhexyl p-methoxycinnamate, 2-ethylhexyl 4-phenylbenzophenone-2', carboxylic acid, 2-ethylhexyl salicylate, glyceryl aminobenzoate

(glyceryl PABA), homosalate, lawsone with dihydroxyacetone (dihydroxyacetone; lawsone (2-hydroxy-1, 4 naphthoquinone)), menthyl anthranilate, 3-(4-methylbenzylidene)-camphor, oxybenzone (benzophenone-3), padimate A (amyl p-dimethylaminobenzoate, amyl para-dimethylaminobenzoate, amyl dimethyl PABA padimate), padimate O (octyl dimethyl PABA), 2-phenylbenzimidazole-5-sulfonic acid, 2-phenylbenzimidazole sulfonic acid), red petrolatum, sodium 3,4-dimethylphenylglyoxylate, 3,4-dimethylphenyl-glyoxylic acid sodium salt, salisobenzene, titanium dioxide, triethanolamine salicylate, and consequently when applied to the body they can stimulate an allergic response.

These active components are usually combined with inactive ingredients which act as a gel, cream, oil or base, for example, alcohol, allantoin, beeswax, benzyl alcohol, BHT, cetyl palmitate, cetyl stearyl glycol, citric acid, close OII, cocoa butter, colour, dimethicone, dimethyl polysiloxane, ethyl alcohol, FD & C yellow No. 5, FD & C red No. 4, fragrances, glycerin, glyceryl stearate, 2-bromo-2-nitropropane-1, 3 diol, camphor, carbomer 934, carboset, cetyl alcohol, paraffin, PEG 2 stearate, petrolatum, polyoxyl-40-stearate, polysorbate 60, propellant 46, propellant 12/114, propylparaben, propylene glycol, propylene glycol stearate, quaternium 15, SD alcohol

40, sesame oil, silica, isopropyl myristate, isopropyl palmitate, lanolin, lanolin alcohol, lanolin derivatives, lanolin oil, menthol, methylparaben, microcrystalline titanium-coated, mica platelets, microcrystalline wax, mineral oil, oleth-3-phosphate, parabens, sodium carbomer, sorbitan oleate, sorbitan stearate, stabilized aloe vera gel, stearyl alcohol, synthetic spermaceti, triethanolamine, triethanolamine stearate, water, wax, zinc oxide.

Although satisfactory protection can be obtained with these synthetic materials, there is a need for agents which afford protection but do not possess the disadvantages of these synthetic chemicals (i.e. allergenicity and toxicity).

Consequently, there has been a move towards discovering naturally occurring but effective U.V. absorbing agents.

GB 87 08448 discloses the use of extracts of hair containing hydrolysed keratin and natural melanin in granular form for use as a screen agent effective in absorbing U.V. radiation thereby preventing damage to tissue. The present invention takes that application further and considers the use of the individual components of such extracts as U.V. absorbing agents in their own rights.

For practical purposes the solar spectrum at the earth's surface consists of wavelengths of electromagnetic energy ranging from between 260 and 1800 nanometres. The sun's rays associated with diseases are related to the light sensitivity range from 260 to 1800 nanometres. The U.V. spectrum lies between 200 and 400 nanometres, visible light between 400 and 770 nanometres and infrared rays beyond 770 nanometres. Ultraviolet radiation from any source can be divided into 3 bands from the longer to the shorter wavelengths as follows:-

1. U.V.-A (Black light radiation, long wavelength U.V. radiation, near U.V. radiation). Wavelength 320 to 400 nanometres. U.V.-A radiation can cause tanning of the skin but is weak in causing reddening of the skin. About 20 to 50 joules/cm² of U.V.-A energy is required to produce a minimally perceptible redness reaction (the minimal erythema dose of MED).

2. U.V.-B (Sunburn radiation, middle U.V. radiation). Wavelength 290 to 320 nanometres. U.V.-B radiation causes sunburn and also stimulates pigmentation in the skin (tanning). Approximately 20 to 30 millijoules/cm² of U.V.-B energy is required to produce 1 MED (about 1,000 times less than the dose of U.V.-A). The action spectrum causing sunburn lies

between 290 and 320 nanometres in the U.V.-B band with a maximum effect at 296.7 nanometres. Whilst the quantity of U.V.-B radiation reaching the earth's surface is small, under optimal environmental conditions for sunburn only 0.2% of the total solar radiation causes erythema of the skin. About 95% of this burning radiation may be absorbed by normal white skin. Different amounts of energy reach the earth's surface at various wavelengths from 290 to 320 nanometres. At 307.4 nanometres the maximal amount of energy to cause sunburn is delivered by the sun to the skin.

3. U.V.-C (Germicidal radiation, short U.V. radiation, far U.V. radiation). Wavelength from 200 to 290 nanometres. Light at 254 nanometres can induce specific forms of DNA damage (pyrimidine dimers) in the basal layer of the living skin. This damage is responsible for cell death when present at high levels and may be mutagenic at low levels. At 280 nanometres (U.V.-C) proteins are efficiently crosslinked to other macromolecules, impairing normal cell behaviour and causing death at high dose levels. U.V.-C radiation from sunlight reaches the earth's surface in small amounts and may be a contributory factor in the induction of skin cancer in pale skinned people living in tropical regions. Epidemiological studies have

indicated a strong correlation between exposure to sunlight and skin cancer amongst Caucasians in Australia, South Africa and the Southern USA. Artificial U.V. sources can emit U.V.-C radiation. U.V.-C radiation is not effective in tanning although it does cause erythema, requiring 5 to 20 millijoules/cm² of energy or produce 1 MED.

In nature, species that are exposed to sunlight have evolved two protective mechanisms.

The first mechanism prevents or reduces damage to skin cells by the production of melanin which has a maximum absorption in the U.V.-B and U.V.-C wavelengths thus protecting against the most harmful rays of the sun whilst allowing the U.V.-A wavelengths also present to stimulate its own production (i.e. tanning).

The second mechanism is the repair of damaged cells by the enzymatic removal of U.V.-C induced pyrimidine dimers from DNA and their replacement with normal nucleotides.

Synthetic chemicals used as active agents protect the skin against U.V. light in an indiscriminating fashion, usually absorbing in both the U.V.-A and U.V.-B wavelengths. Whilst this affords some degree of protection, it fails to promote the natural tanning process. Furthermore, these chemicals are relatively

small molecules and are able to pass through the protective barrier of the skin (stratum corneum) and into the blood, where in some individuals allergic responses may be induced. A further disadvantage of these synthetic chemicals is their instability in sunlight, results in the formation of breakdown products that may have toxic properties. In contrast melanin granules are very stable in the presence of U.V. irradiation and do not break down.

Sun protection products reduce by varying amounts the solar radiation reaching the skin and thereby affect the physiological response and extent of the erythema reaction produced for any given exposure. These products have a number of mechanisms by which they effect their screen function. These comprise absorbing, reflecting or scattering the harmful burning rays of the sun.

Having analysed the problem further, it became apparent that the preferred material should a) absorb U.V. radiation having wavelengths in the region of 200 to 290 nanometres (i.e. U.V.-B and U.V.-C) and b) allow radiation in the 320 to 400 nanometres range to reach the skin surface, since radiation of this range (U.V.-A) induces tanning with a minimal risk of skin damage.

It has been found that natural melanin granules absorb U.V. in the above regions and furthermore are large enough not to be absorbed into the blood stream when applied with a carrier to the skin and consequently initiate an allergic response.

Synthetic melanin-like products, produced for example, by persulphate oxidation of tryptophan have been found not to have such properties.

It has further been discovered that the isolation of melanin from melanin containing substrates such as hair, and skin by the use of alkaline reagents such as sodium hydroxide or sodium sulphide does not effect the U.V. absorbing properties of the molecule. Thus, whilst many proteins are normally denatured and inactivated by heat and alkaline reagents such as sodium hydroxide or sodium sulphide, the isolation of naturally occurring melanin by the use of such reagents does not effect the U.V. absorbing properties of the protein.

According to one aspect of the present invention there is provided an agent for absorbing U.V. radiation which comprises melanin.

According to another aspect of the invention there is provided a pharmaceutical preparation for use as a filter of U.V. radiation which preparation comprises melanin.

Preferably, the melanin source is in the form of natural melanin granules, and more preferably still contains the pigment eu-melanin as opposed to phaeo-melanin.

More preferably still the melanin granules are at least 1 micron in diameter.

In one embodiment, the melanin is obtained from human hair, although other melanin containing substrates such as animal hair, human or animal skin, could be used.

According to another aspect of the invention there is provided a method of preparing a preparation for use as an agent for absorbing U.V. radiation which method comprises the steps of alkali hydrolyzing a melanin containing source, neutralizing the product of hydrolysis with acid and separating the melanin granules from the other hydrolysis products. Preferably separation is conducted by centrifugation.

In one embodiment the melanin granules are isolated by chemical means such as treatment and mild heating with sodium sulphide, although alkali hydrolysis with other agents such as sodium hydroxide could be used. The advantage of sodium sulphite over sodium hydroxide is that after hydrolysis less acid is required to neutralise the product.

Preferably the melanin isolated will comprise melanin granules having a diameter in the order of 1 micron since these granules and agglomerates thereof are too large to pass through the skin barrier and hence will not pass into the blood. Consequently, they are unable to produce an immune reaction.

One advantage of using melanin is that even if granules of melanin should gain access to the blood through broken skin they will be non-toxic, since melanin is phylogenetically conserved and is, therefore, unlikely to produce an immune reaction.

In another embodiment the melanin granules are incorporated into or sandwiched between a carrier such as glass or plastics, so that structures can be produced which absorb U.V. light.

In yet a further embodiment the carrier may be a transporter medium such as paint, so that the agent can be used as a coating medium.

It has also been found that keratin and/or the hydrolysis products thereof act as an effective U.V. absorbing material.

Since the hydrolysis products of keratin consist of polypeptides and amino acids it is possible to selectively remove the smaller molecules so that peptides too large to pass through the skin are left, thereby providing a product which can be used that will not illicit an allergic response.

According to another aspect of the present invention there is provided an agent for absorbing U.V. radiation which agent comprises keratin and/or the hydrolysis products thereof.

Preferably, the hydrolysis product is subjected to sorting so that molecules with a size too large to pass through the skin are preferentially selected. Such separation means could include the use of chromatography although other purification procedures could be used.

In one embodiment the keratin and hydrolysis products thereof are obtained by hydrolysis of a keratin source, for example human or animal skin or hair. Preferably, the skin or hair is subjected to alkali hydrolysis using an alkaline reagent, for example sodium sulphide or sodium hydroxide at elevated temperature.

In another embodiment the keratin, and more particularly the polypeptides obtained therefrom by hydrolysis could be made synthetically using a peptide synthesiser.

In another embodiment the keratin and/or hydrolysis products thereof are incorporated into a carrier for use as a pharmaceutical product.

In another embodiment the keratin and/or hydrolysis products thereof are incorporated into or

sandwiched between a carrier such as glass or plastics, so that structures can be produced which absorb U.V. light.

In yet a further embodiment the carrier may be a transporter medium such as paint so that the agent can be used as a coating medium.

According to one aspect of the present invention there is provided a pharmaceutical preparation for use as a filter of U.V. radiation which preparation comprises keratin and/or the hydrolysis products thereof.

According to another aspect of the present invention there is provided a method of preparing a preparation for use as an agent for absorbing U.V. radiation, which method comprises the steps of alkali hydrolyzing a keratin containing source, neutralizing the product of hydrolysis with acid and separating the granules, keratin and/or the larger hydrolysis products thereof from the other hydrolysis products by centrifugation.

A specific embodiment of the invention will now be described, by way of example only, with reference to the drawings, in which:-

Fig.1 illustrates the absorption achieved by differing compositions of differing wavelength. Solution (a) is a 100×10^{-6} % w/w solution of keratin and hydrolysing product thereof in water; solution (b) is a 79.9×10^{-3} % w/w solution of melanin in water,

and solution (c) is a 0.08% w/w solution of keratin and hydrolysing product thereof and melanin in solution.

Fig.2 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 310nm (U.V.B)

the hydrolate being produced using sodium sulphide as the hydrolysing agent.

Fig.3 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 310nm (U.V.B)

the hydrolate being produced having sodium hydroxide as the hydrolysing agent.

Fig.4 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 280nm (U.V.C)

the original hydrolysate being produced using sodium sulphide as the hydrolysing agent.

Fig.5 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 280nm (U.V.C)

the original hydrolysate being produced using sodium hydroxide as the hydrolysing agent.

Fig.6 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 340nm (U.V.A)

the original hydrolysate produced using sodium sulphide as the hydrolysing agent, and

Fig.7 illustrates the active concentration ranges of:-

- a) a solution of keratin and its hydrolysis products
- b) a solution of melanin in water
- c) a solution of reconstituted hydrolate product by adding a) to b) at 340nm (U.V.A)

the original hydrolysate produced using sodium hydroxide as the hydrolysing agent.

A hydrolysate was prepared by the degradation of 4g of hair with a 0.5 molar sodium sulphide solution at 65°

for 24 hours and the resulting suspension was neutralised with hydrochloric acid.

An advantage of this method is that hydrolysis with alkali is an extremely destructive procedure which means that macromolecules such as proteins, enzymes and nucleic acids are reduced to simple molecules such as amino acids and nucleotides, leaving only the melanin granules remaining intact after extraction. Thus, most micro-organisms are destroyed, and the alkaline environment ensures that even bacterial spores, which might normally survive high temperatures, are degraded. Viruses, bacteria, genetic material (e.g. chromatin, DNA, RNA and any functional gene sequences) or enzymes necessary for cellular function would also all be destroyed. Consequently this procedure avoids the danger of transmitting health hazards.

The resulting 4% w/w solution, i.e. a solution containing 4g of active agent (melanin and hydrolysed keratin and amino acids) per 100g of solution, contained approximately 50 micrograms melanin granules (0.005% w/w), the remainder consisting mainly of hydrolysed keratin and amino acids (about 3.995% w/w).

This 4% w/w solution was diluted to various concentrations and tested. A 0.4% w/w solution was shown to absorb all the incident U.V. light (zero transmission at wavelengths between 340 and 240 nanometres). A small amount, (around 2% transmission) of U.V. Light was shown to pass through the solution between 340 and 350 nanometres (U.V.-A).

Thus further dilutions were made and the effect of concentration of each component at the different wavelengths, i.e. UVA, UVB and UVC was noted. The results of these experiments are shown tabulated to Figures 2 to 7.

Where a w/w value for the hydrolysate is given, an equivalent value is shown for the individual components. Thus for example, a 0.08% w/w for the hydrolysate (i.e. melanin and keratin and the hydrolysing product thereof) is equivalent to a 100×10^{-6} % w/w melanin concentration or a 79.9×10^{-3} % w/w keratin and the hydrolysing product thereof.

At 0.08% w/w the hydrolysate gave a result showing the solution absorbed 90% of the U.V.-C wavelength, around 80% U.V.-B wavelength and 65% U.V.-A wavelengths. This indicated that the hydrolysate was more efficient at removing the harmful wavelengths (i.e. U.V.-B and U.V.-C) than the tanning wavelengths (U.V.-A).

Further investigation on the 0.08% w/w hydrolysate was conducted to see the effect of the individual components on U.V. absorption.

The 0.08% w/w hydrolysate formed was consequently subjected to centrifugation at 5,000g for a time period suitable to separate the melanin granules from the supernatant comprising mainly hydrolysed keratin and amino acids.

The pellet obtained was resuspended in a volume of water equivalent to that of the starting volume and the 100×10^{-6} % w/w melanin suspension was investigated to see the absorbance at various wavelengths. These results are shown in Fig.1.

It can be seen from Fig. 1 that melanin gave a transmission spectrum which ranged from 65% absorbance at 240 nanometres to 40% absorbance at 350 nanometres.

In contrast, the transmission spectrum of the soluble component comprising mainly hydrolysed keratin and amino acids (79.9×10^{-3} % w/w) selectively absorbed 85% of the U.V.-C wavelengths, 70 to 40% of the U.V.-B wavelengths and 30 to 40% of the U.V.-A wavelengths.

These results compare less favourably than those achieved when the hydrolysate was reconstituted by adding back the melanin granules but each system could be more favourable under certain conditions. For example, the use of melanin on its own could have advantages where the recipient of a cosmetic containing the U.V. absorbing material is prone to allergic response.

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CLAIMS

1. An agent for absorbing U.V. radiation which comprises melanin.
2. An agent as claimed in claim 1, wherein the melanin is in the form of natural melanin granules.
3. An agent as claimed in claim 1 or 2, wherein the melanin comprises eu-melanin.
4. An agent as claimed in claim 3, wherein the melanin granules are greater than 1 micron in diameter.
5. An agent as claimed in any of the preceding claims wherein the agent is combined with a carrier.
6. An agent as claimed in claim 5, wherein the carrier is a gel, a cream or an oil.
7. An agent as claimed in claim 5, wherein the agent is incorporated into said carrier or sandwiched between a carrier, which carrier should transmit e.m. radiation in the region of 200 to 400 nm.
8. An agent as claimed in claim 7, wherein said carrier capable of transmitting e.m. radiation in the region of 200 to 400 nm is glass, plastics or a transporter medium.
9. An agent as claimed in claim 6, wherein the melanin is present in the range 500×10^{-6} % w/w to 50×10^{-6} % w/w weight of melanin to the total weight of the agent and carrier.
10. An agent as claimed in claim 9, wherein the amount of melanin present is 100×10^{-6} % w/w of melanin to the total weight of the agent and carrier.

11. A pharmaceutical preparation for use as a filter of U.V. radiation, which preparation comprises melanin.

12. A pharmaceutical preparation as claimed in claim 11, wherein the melanin is in the form of natural melanin granules.

13. A pharmaceutical preparation as claimed in claim 11, wherein the melanin is in the form of Eu-melanin.

14. A pharmaceutical preparation as claimed in claim 12, wherein the natural melanin granules are at least 1 micron in diameter.

15. A pharmaceutical preparation as claimed in claims 11 to 14, which further comprises a carrier material.

16. A pharmaceutical preparation as claimed in claim 15, wherein the carrier is in the form of a gel, cream or oil.

17. A pharmaceutical preparation as claimed in claim 16, wherein the melanin present in the range 500×10^{-6} % w/w to 50×10^{-6} % w/w weight of melanin to the total weight of the agent and carrier.

18. A pharmaceutical preparation as claimed in claim 16, wherein the amount of melanin present is 100×10^{-6} % w/w of melanin to the total weight of the agent and carrier.

19. A method of preparing the agent or pharmaceutical preparation claimed in claims 1 to 18, which comprises the steps of alkali hydrolyzing a melanin containing source, neutralizing the product of hydrolysis with acid and separating the melanin granules from the other hydrolysis products.

20. A method as claimed in claim 19, wherein the melanin source is skin or hair.

21. A method as claimed in claim 19, wherein the alkali hydrolysing agent is sodium sulphide or sodium hydroxide.

22. An agent for absorbing U.V. radiation which comprises keratin and/or the hydrolysis products therefor.

23. An agent as claimed in claim 22, wherein the said keratin or hydrolysis products thereof are subjected to purification so that amino acids and small peptides are removed.

24. An agent as claimed in claim 22, wherein the keratin or hydrolysis products thereof are synthesised.

25. An agent as claimed in claims 22 to 24 wherein the agent is combined with a carrier.

26. An agent as claimed in claim 25, wherein the carrier is a gel, a cream or an oil.

27. An agent as claimed in claim 25, wherein the agent is incorporated into said carrier or sandwiched

between a carrier, which carrier should transmit e.m. radiation in the region of 200 to 400 nm.

28. An agent as claimed in claim 27, wherein said carrier capable of transmitting e.m. radiation in the region of 200 to 400 is glass, plastics or a transporter medium.

29. An agent as claimed in claims 5 and 6, wherein the keratin or hydrolysis products thereof is present in the range 3.995×10^{-3} % w/w to 39.95×10^{-3} % w/w weight of keratin and/or hydrolysis products thereof to the total weight of the agent and carrier.

30. An agent as claimed in claim 29, wherein the amount of keratin or hydrolysis products thereof present is 79.9×10^{-3} % w/w of keratin or hydrolysis product thereof to the total weight of the agent and carrier.

31. A pharmaceutical preparation for use as a filter of u.v. radiation, which preparation comprises keratin and/or the hydrolysis products thereof.

32. A pharmaceutical preparation as claimed in claim 31, which further comprises a carrier material.

33. A pharmaceutical preparation as claimed in claim 32, wherein the carrier is in the form of a gel, cream or oil.

34. A pharmaceutical preparation as claimed in claim 33, wherein the amount of keratin and/or

hydrolysis product thereof is present in the range 399.5×10^{-3} % w/w to 39.95×10^{-3} % w/w weight of keratin and/or the hydrolysis product thereof to the total weight of the agent and carrier.

35. A pharmaceutical preparation as claimed in claim 34, wherein the amount of keratin and/or hydrolysis product thereof is 79.9×10^{-3} % w/w keratin or hydrolysis product thereof to the total weight of the agent and carrier.

36. A method of preparing the agent or pharmaceutical preparation claimed in claims 22 to 35, which comprises the steps of alkali hydrolyzing a keratin containing source, and neutralizing the product of hydrolysis with acid and separating the keratin and hydrolysis products thereof from the other components of the supernatant.

37. A method as claimed in claim 36, wherein the keratin source is skin or hair.

38. A method as claimed in claim 32, wherein the alkali hydrolysing agent is sodium sulphide or sodium hydroxide.

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